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ORIGINAL ARTICLE

Knee extensor muscle oxygen consumption in relation to muscle activation

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Abstract Recently, fatigability and muscle oxygen consumption (mVO_2) during sustained isometric contractions were found to be less at shorter (30° knee angle; 0° = full extension) compared to longer knee extensor muscle lengths (90°) and, at low torques, less in the rectus femoris (RF) muscle than in the vastus lateralis and medialis. In the present study we hypothesized that these findings could be accounted for by a knee angle- and a muscle-dependent activation respectively. On two experimental days rectified surface EMG (rsEMG) was obtained as a measure of muscle activation in nine healthy young males. In addition, on day 1 maximal torque capacity (MTC) was carefully determined using superimposed nerve stimulation on brief high intensity contractions (> 70%MVC) at 30, 60 and 90° knee angles. On day 2, subjects performed longer lasting isometric contractions (10–70%MTC) while mVO_2 was measured using near-infrared spectroscopy (NIRS). At 30°, maximal mVO_2 was reached significantly later (11.0 s ± 6.5 s) and was 57.9 ± 8.3% less (average ± SD, across intensities and muscles) than mVO_2 at 60 and 90° ($p < 0.05$). However, rsEMG was on average only 18.0 ± 11.8% ($p = 0.062$) less at the start of the contraction at 30°. At 10%MTC at all knee angles, maximal mVO_2 of the RF occurred significantly later (28.8 ± 36.0 s) and showed a significantly smaller increase in rsEMG compared to both vasti. In conclusion, it is unlikely that the tendency for less intense muscle activation could fully account for the ~60% lower oxygen consumption at 30°, but the later increase in RFmVO_2 seemed to be caused by a less strong activation of the RF.

Keywords Activation · Muscle length · Muscle oxygen consumption

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Introduction

During daily activities different muscles are used over a whole range of muscle lengths. When activating skeletal muscle, work is performed by the muscle and concomitantly it consumes oxygen. Thus, it is very relevant to investigate both muscle activation and muscle oxygen consumption during in vivo muscle activity at different muscle lengths. Knee extensor endurance is greater at extended (short muscle length) compared to flexed (long muscle length) knee angles during sustained isometric contractions to exhaustion, even at the same relative contraction intensity (Hisaeda et al. 2001; Kooistra et al. 2005; Ng et al. 1994; Place et al. 2005). One reason for this difference could be a difference in maximal voluntary activation between knee angles (de Ruiter et al. 2004; Suter and Herzog 1997). When relative contraction intensities between knee angles are compared, normalizing to the most accurate maximal value at each knee angle is important. If the maximal voluntary contraction (MVC) is used as a gold standard, and differences in maximal voluntary activation

between knee angles occur, differences in the relative contraction intensities between those knee angles will mistakenly be created. Therefore, contraction intensities are better expressed as a percentage of the maximal torque capacity (MTC) rather than MVC. It has further been shown that the difference in endurance (time to torque failure) between knee angles does not originate from differences in central drive at the point of torque failure or from differences in muscle perfusion (Kooistra et al. 2005). However, potentiation, the enhancement of contractile response as a consequence of prior activation, has been suggested to contribute to the increased endurance at extended compared to flexed knee angles (Place et al. 2005) as potentiation is greater at short compared to long muscle length in rats (Rassier et al. 1997; Rijkelijkhuizen et al. 2005). Furthermore, potentiation may lead to a constant force output despite a decline in motor unit firing rate (Klein et al. 2001). Therefore, a muscle length-dependent potentiation could allow for a slower increase in muscle activation at extended compared to flexed knee angles during constant force production. This less intense muscle activity may postpone the recruitment of large fatigable motor units, which may account for the lower muscle oxygen consumption (mVO_2) found previously at extended compared to flexed knee angles (de Ruiter et al. 2005). It would therefore be very interesting to examine muscle activation and oxygen consumption over a range of relative contraction intensities at extended compared to flexed knee angles. In addition, insight into potential mechanisms limiting endurance at different muscle lengths found previously (Hisaeda et al. 2001; Kooistra et al. 2005; Ng et al. 1994; Place et al. 2005) may be gained by the simultaneous investigation of muscle activation and oxygen consumption.

Particularly at low contraction intensities there are indications for differences in the muscle activation of the rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) muscle. Using EMG, the activity of synergistic motor neuron pools has been shown to alternate during low-force sustained contractions by which constant force production is maintained (Kouzaki et al. 2002; Tamaki et al. 1998). In addition, the change in root mean square EMG of the VL and VM was twofold greater compared to the RF during sustained low force contractions (Ebenbichler et al. 1998). These variations may arise from the unique role of bi-articular muscles (such as the RF muscle) in controlling the distribution of net moments about the joints (Jacobs and van Ingen Schenau 1992). In addition, considering the lower mVO_2 of the RF compared to VL and VM at low contraction intensities (de Ruiter et al. 2005), an accompanying less intense muscle activation (lower EMG activity) of the RF compared to the VL and VM would be expected.

The first aim of this study was to investigate whether less muscle activation, measured with rectified surface EMG (rsEMG), could account for the lower muscle oxygen utilization at extended compared to flexed knee angles. We hypothesized that at the same relative torque, relative rsEMG would be consistently lower at extended compared to flexed knee angles and similarly, a lower muscle oxygen consumption was expected at the extended knee angle. Secondly, we investigated if differences in muscle activation between the knee extensor group muscles were related to similar differences in muscle oxygen utilization, particularly at low contraction intensities. We hypothesized that at low contraction intensities a relatively lower oxygen consumption of the RF would be accompanied by relatively less rsEMG of the RF compared to the VL and VM.

Methods

Subjects

Thirteen healthy male subjects (mean ± SD age of 25.4 ± 3.1 years) volunteered to be subjects for this investigation. Before participation, each subject was thoroughly informed about the procedures and provided written informed consent prior to testing. Only subjects capable of maximal voluntary activation levels > 90% at each knee angle (see below) were included in the present study. The study was performed according to the Declaration of Helsinki and approved by the local ethics committee. Subjects did not to perform any fatiguing exercise 48 h prior to measurements.

Experimental procedures

The subjects visited our laboratory on three occasions with at least 2 days in between. The first visit was a habituation session; subjects were familiarized with the set-up, electrical stimulation, and stable constant isometric knee extension with a pressure cuff and visual feedback. The first experimental day (day 1) was used to accurately determine maximal torque capacity (MTC, see below) and record surface EMG of the RF, VL, and VM at high (> 70% MVC) contraction intensities. During the second experimental day (day 2), muscle oxygen uptake (mVO_2) and surface EMG of the same muscles were recorded at extended (30°, 0° = full knee extension) and flexed (60° and 90°) knee angles at

several submaximal intensities (10, 30, 50, 70%MTC) as well as during two sustained maximal attempts.

Torque measurements

Isometric knee extension torque of the right leg was measured using a custom-built dynamometer. For details see Kooistra et al. (2005), but briefly, subjects were seated with their hips at 70° (0° = full extension) and shoulders, hips, and lower thigh were strapped to the dynamometer. Knee joint angles were determined with a handheld goniometer (model G300, Whitehall Manufacturing) and the dynamometer arm was positioned so that the indicated knee angles were angles with subjects delivering ~50% of maximal torque. Real-time force applied to the force transducer (KAP, E/200 Hz, Bienfait B.V. Haarlem, The Netherlands, range 2,000 N) was displayed on line on a computer monitor and digitally stored (1 kHz) on computer disc. The force signals were automatically corrected for gravity at each angle: the average force applied by the weight of the limb to the transducer during the first 50 ms after the start of a recording, with the subject seated in a relaxed manner, was set to zero force by the computer program. The distance from the knee axis to the centre of the force transducer was determined for each subject to enable torque calculation.

Electrical stimulation

A cathode (self-adhesive stimulation electrode, 5 × 5 cm, Schwa-Medico, The Netherlands) was placed over the femoral nerve. The anode (13 × 8 cm) was placed over the gluteal fold. The quadriceps femoris muscle was stimulated transcutaneously with rectangular pulses of 100μs using a computer-controlled constant current stimulator (Digitimer DS7H, Digitimer Ltd., Welwyn Garden City, UK). Stimulation current was increased until torque measured in response to a triplet (three 100 μs pulses applied at 300 Hz) levelled off at each knee angle tested. The current (in mA) was then increased by a further 50 mA to ensure supramaximal stimulation (range 250–600 mA). It was assumed that at this point all muscle fibres of the knee extensors were activated. Usually torque generated by the triplet is about ~35% of MTC (Kooistra et al. 2005).

Maximal voluntary contraction (MVC) and maximal torque capacity (MTC)

Since muscle oxygen consumption (mVO_2, see below) and rsEMG (see below) during constant torque production at fixed percentages of MTC were obtained on day 2, we considered it very important to have highly accurate values for MTC. MTC was preferred to MVC as a 100% value because it eliminates the potential effect of knee angle-dependent differences in maximal voluntary activation. For example, when maximal voluntary activation is less than 100% during an MVC, MVC underestimates the MTC of the muscle. When differences in maximal voluntary activation occur between knee angles (de Ruiter et al. 2004), using MVC as a 100% value may falsely produce differences in mVO_2 and EMG when torque is set as a percentage of MVC rather than MTC, between knee angles.

During day 1, MVC torque and MTC were determined at three knee angles (30°, 60° and 90°). Subjects were asked to maximally generate isometric torques for about 3–4 s to determine MVC extension torque. Real-time torque was visible on a computer monitor and subjects were vigorously encouraged to exceed their maximal value, which was also displayed. MVC torque was determined as the highest stable 1,000 ms part of the force signal multiplied by the subjects' moment arm. MVC torque was taken as the highest value, which did not exceed preceding attempts by > 5%, allowing a maximum of four attempts. To quantify MTC as accurately as possible 5–7 voluntary contractions (70–100%MVC) were performed with a superimposed triplet. The contractions were performed in random order across knee angles separated by three minutes rest. The increment in torque as a result of the superimposed triplet was plotted as a function of the stable torque plateau reached just before the superimposed stimulation. Following linear regression, the intersection with the *x*-axis yielded MTC (Fig. 1). At MTC, all muscle fibres were considered maximally active. The level of voluntary activation was defined afterwards as MVC/MTC × 100. Surface EMG of the RF, VL, and VM muscle were recorded during each contraction.

Surface electromyography

Electromyographic activity of the RF, VL, and VM muscle was recorded using surface EMG electrodes (Blue Sensor, Ambu, Ølstykke, Denmark, lead-off area 1.0 cm^2). After shaving, roughening and cleansing the skin with 70% ethanol electrodes were placed on the muscle belly in a bi-polar configuration, in line with the muscle fibre direction, with an inter-electrode distance of 25 mm. Reference electrodes were placed on bone structures, on each patella and on the lateral epicondyle of the femur of the right leg. The location of each electrode was accurately marked with a waterproof felt tip pen for precise electrode re-application in subsequent sessions. Surface EMG signals were amplified

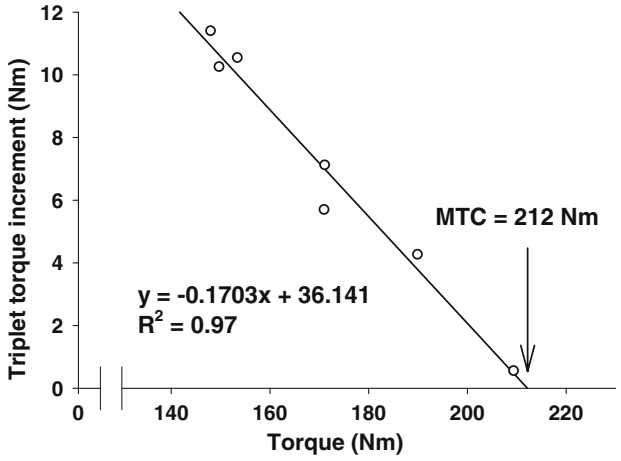

Fig. 1 An example for one subject at the 30° knee angle. The torque increment as a result of the triplet on a voluntary contraction shown as a function of the delivered torque just before the triplet (*x*-axis). Using linear regression, the intersection at the *x*-axis is calculated (*black arrow*), and it was defined as the maximal torque capacity (MTC)

(100 ×), digitised (1 kHz), and stored with the force signal on computer disc. All EMG signals were band-pass filtered (10–400 Hz). RsEMG amplitude was calculated for the RF, VL and VM for 1,000 ms segments just before the superimposed stimulation on day 1; on day 2 rsEMG was calculated at torque onset as well as 500 ms before and after mVO_2 was measured (see below). rsEMG values were normalized to those obtained during the highest MVC on day 1.

Near-infrared Spectroscopy (NIRS)

On day 2, oxygen consumption of the RF, VL and VM muscle was determined during isometric knee extension contractions by use of a continuous-wave near-infrared spectrophotometer (Oxymon, Artinis Medical Systems, Zetten, The Netherlands), which generated light at 780 and 850 nm (van der Sluijs et al. 1998) and was regularly calibrated by Artinis Medical Systems. The three optode sets were each fixed in a mould with an inter-optode distance of 45 mm (penetration depth of the tissue was therefore 22.5 mm). The moulds were secured to the upper leg with elastic Velcro straps such that the optodes did not move during contraction. The optodes were positioned over the centre of the muscle bellies. Note that the surface EMG electrodes were placed in between the NIRS optodes to ensure that the recording volume of the electrodes coincided with that of the NIRS optodes.

With NIRS, the tissue oxygenation level can be measured non-invasively, in fact, the optical change in density of the tissue is measured, which, by modification of the Lambert–Beer law (Livera et al. 1991), can be transformed into the change in concentrations of oxyhaemoglobin ([O_2Hb]) and -myoglobin ([O_2Mb]) and deoxyhaemoglobin and -myoglobin ([HHb] and [HMb]). Due to the overlap in the spectrum, haemoglobin and myoglobin cannot be measured separately; O_2Hb and HHb will, respectively, denote the oxygenated and deoxygenated form of both proteins in the present study. The rates of concentration changes of O_2Hb and HHb, or the slopes (Fig. 2) of the [O_2Hb]- and [HHb]-time curves, represent the oxygen consumption per unit time in the muscle (mVO_2, see also (Grassi et al. 2003)) Blood volume and total Hb (the sum of [O_2Hb] and [HHb]) of the m. quadriceps were kept constant by inflation of a pressure cuff (Hokanson SC 10D), which was placed around the most proximal part of the thigh, a few seconds prior to each contraction and deflated a few seconds afterward (for details see (de Ruiter et al. 2005)). The maximal slopes of the [O_2Hb]- and [HHb]-time signals (which were not statistically different in any of the muscles at any of the knee angles) were averaged, and this absolute value, mVO_{2max}, was calculated at all contraction intensities. Furthermore, mVO_2 was expressed relative to maximal deoxygenation (% s^{-1}), which is standard procedure (de Ruiter et al. 2005). By expressing mVO_2 in this manner, potential differences in subcutaneous fat between muscles, which greatly affect NIRS measurement sensitivity (Niwayama et al. 2000; van Beekvelt et al. 2001), are accounted for. Maximal deoxygenation is defined as the absolute difference in [O_2Hb] (and [HHb]) when virtually all O_2Hb is converted into HHb. In this study, maximal deoxygenation was determined at the 60° knee angle in rest as it was demonstrated previously to be independent of torque level (0–100%MVC) and knee angle (de Ruiter et al. 2005). NIRS-data were sampled at 10 Hz and stored on computer disc.

Due to the greater endurance at the 30° knee angle, at each intensity after torque onset, the maximal slope of the HHb and HbO_2 signals (mVO_{2max}) was expected to be reached at a later point in time (t_{max}) at 30° compared to 60 and 90° knee angles. For an appropriate comparison of mVO_2 and rsEMG between knee angles, the instance at which they are compared should be the same (Fig. 2). Hence, for each intensity, when mVO_{2max} was reached at any muscle, at any of the knee angles, mVO_2 and rsEMG were determined for all muscles at all three knee angles (30, 60 and 90°) for this point in time.

EMG measurements and NIRS

On day 2 isometric knee extensions were performed at the three knee joint angles at 10, 30, 50, and 70%MTC

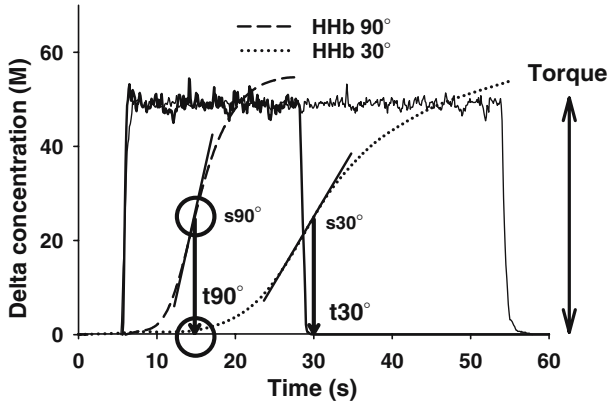

Fig. 2 Near-infrared spectroscopy (NIRS) signals of the vastus medialis (VM) muscle during constant torque production at the 90 (*solid lines***trace**) and 30° (*solid lines* trace) knee angle. The 60° knee angle is not shown for reasons of clarity. Changes in concentration of deoxygenated haemoglobin (HHb, HbO_2 [mirror image of HHb] is not displayed for reasons of clarity) are shown during constant torque production at 30% maximal torque capacity for the 90° (*dashed lines* trace) and 30° (*dotted lines* trace) knee angle. Maximal oxygen consumption (mVO_{2max}) at each angle occurred at the time (t30° and t90°) when the slopes (s30° and s90°) were maximal and is denoted by the two *arrows*. In this example, muscle oxygen consumption (mVO_2) and EMG were measured at t = 15 s (*circles*) for both 90 and 30° knee angles

during which rsEMG and mVO_2 were obtained. The [O_2 Hb]– and [HHb]–time signals of each muscle were displayed online and each contraction was continued past the time at which the maximal slope (mVO_{2max}) of each muscle was reached.

Although we realized maximal voluntary activation might be lower during a sustained MVC (Schillings et al. 2005) compared to the brief MVCs performed on day 1, we wanted two sustained maximal attempts, where the highest stable torque plateau was used for further analysis. The order of the contraction intensities was randomised, but per subject, the same order of intensities was used at each knee joint angle. All subjects started with a sham measurement at 50%MTC, which was used as warm up. Pilot experiments had shown that the mVO_2 of the first measurement was consistently lower than when the same measurement was repeated. Yet, all measurements thereafter regardless of angle or intensity were very reproducible (that is the difference in mVO_2 of two consecutive measurements at the same intensity was <5%). The subjects received 6 min rest between the trials.

Statistics

All results are presented as mean ± SD. Separate repeated measures analyses of variances (ANOVA, SPSS version 12.0) were used to compare the level of voluntary activation and MTC between the three knee angles tested.

With respect to t_{max}, mVO_2, and rsEMG an "overall" ANOVA for repeated measures was performed that included the data obtained at the 10–70%MTC intensity levels. For significant effects in this overall analysis, subsequent ANOVAs for repeated measures were performed to test for any differences between knee angles as well as differences between muscles.

For the data obtained at 10%MTC, an "overall" ANOVA for repeated measures was performed with respect to t_{max}, mVO_2, and rsEMG. Bonferroni tests were used for all post hoc analysis. The level of significance of all statistical analyses was set at $P < 0.05$.

Results

During the familiarization session four subjects did not reach >90% voluntary activation and were excluded from further participation, the results and statistics of nine subjects are presented.

Voluntary activation, MTC and torque

Linear extrapolation of the extra torque as a result of the electrical stimulation on high intensity contractions (>70%MVC) provided high R^2 values at each knee angle (30°: 0.96 ± 0.03; 60°: 0.96 ± 0.03, and 90°: 0.97 ± 0.03), which did not differ between knee angles. The determination of MTC was very accurate since voluntary activation levels were very high, with no differences between knee angles. MTC was significantly ($P < 0.05$) greater at the 60° knee angle compared to the 30 and 90° knee angle, whereas there were no differences between the 30 and 90° knee angle (Table 1).

As expected, during the sustained maximal contraction, maximal voluntary activation at 60° (83.1 ± 11.7%) and 90° (74.4 ± 7.1%) knee angles was significantly lower than the voluntary activation during the brief MVCs executed on day 1 (60°: 95.6 ± 2.8 and 90°: 97.1 ± 2.9%). However and to our surprise, maximal voluntary activation at the 30° knee angle (96.2 ± 3.8%) was very high and not significantly

Table 1 Maximal torque capacity (MTC) and voluntary activation

Knee angle	30°	60°	90°
Voluntary activation (%)	95.6 ± 3.5	95.6 ± 2.8	97.1 ± 2.9
MTC (Nm)	196.0 ± 40.7	273.6 ± 63.4*	187.6 ± 29.2

All values are means ± SD.* denotes significantly different from 30 and 90° knee angles, $P < 0.05$, $n = 9$

different from day 1 (95.6 ± 3.5%). Due to the significant difference in maximal voluntary activation between knee angles, further comparisons of m$\dot{V}O_2$ and muscle activation between the knee angles will be limited to the 10–70%MTC range. At each knee angle, during the sustained contractions at 10, 30, 50 and 70%MTC, average torque was not significantly different from the intended values.

Muscle oxygen consumption between knee angles

As expected, m$\dot{V}O_{2max}$ increased with intensity and, at each intensity, was significantly lower at the 30° knee angle compared to the 60 and 90° knee angle (Fig. 3). At all intensities, t_{max} (the time before m$\dot{V}O_{2max}$ was reached) was significantly greater at 30° compared to 60° and 90° knee angles (Table 2).

To permit an appropriate comparison between m$\dot{V}O_2$ and rsEMG between knee angles, in the present study m$\dot{V}O_2$ was also measured at the same point in time for all muscles and angles per intensity. At the 30° knee angle, in line with our 1st hypothesis, m$\dot{V}O_2$ was significantly lower compared to the 60° and 90° knee angle (Fig. 4). When the m$\dot{V}O_2$ of 60 and 90° was averaged and set to 100% for each intensity level; the m$\dot{V}O_2$ at 30°, across all intensity levels, for RF, VL, and VM was only 47.4 ± 10.2, 45.3 ± 8.5, and 33.7 ± 8.6%, respectively.

Muscle activation between knee angles

In comparing the level of muscle activation between knee angles (with regard to our first hypothesis), we felt that the rsEMG at the start of the contraction would represent any initial potential difference in demand between the respective knee angles tested. Surprisingly, and in contrast to muscle oxygen consumption, rsEMG at the start of the contraction was not different between knee angles (Fig. 5.). However, there was a trend for a main effect (when all intensities (10–70%MTC) were included) for knee angle ($p = 0.062$). Expressed as a percentage of the rsEMG of 60° and 90° (averaged across all intensity levels), rsEMG levels at 30° were: 86.5 ± 15.4, 80.1 ± 13.1, and 79.4 ± 12.9% for the RF, VL, and VM muscle, respectively. The rsEMG values obtained at the same time point as m$\dot{V}O_2$ was reached were comparable ($P > 0.05$) to those measured at the start of the contraction and not different ($P > 0.05$) between knee angles. At the time point m$\dot{V}O_2$ was measured, rsEMG levels at 30° were: 84.8 ± 14.7, 89.5 ± 13.1, and 85.9 ± 13.2% for the RF, VL and VM muscle, respectively, expressed as a percentage of 60 and 90° knee angles.

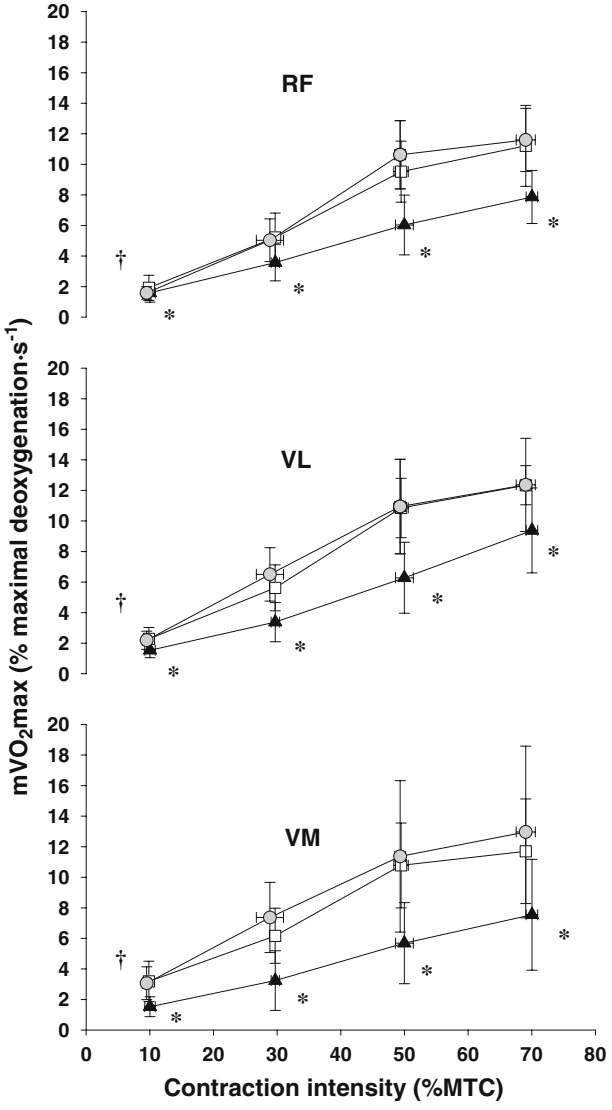

Fig. 3 Maximal muscle oxygen consumption (m$\dot{V}O_{2max}$) for the rectus femoris (RF, top panel), vastus lateralis (VL, mid panel) and vastus medialis (VM, bottom panel) muscle at 30° (*black triangles*), 60° (*white squares*) and 90° (*gray circles*) knee angles at different contraction intensities (10–70%MTC).* m$\dot{V}O_{2max}$ significantly lower at the 30° compared to the 60° and 90° knee angle. † Significant main effect of muscle at 10%MTC, $P < 0.05$

Muscle oxygen consumption and muscle activation at 10%MTC

We will now focus on probable differences in m$\dot{V}O_2$ and rsEMG at 10%MTC (our second hypothesis). With respect to m$\dot{V}O_{2max}$ there was a significant main effect of muscle with further post-hoc testing showing no differences between muscles. Furthermore, the time before m$\dot{V}O_{2max}$ was reached (t_{max}) for the RF was significantly greater than that for the VL and VM muscle. The extent to which this occurred was substantial; t_{max}

Table 2 Time to maximal muscle oxygen consumption (t_{max}) of the RF, VL, and VM at different contraction intensities and knee angles

Muscle	Knee angle	Contraction intensity (%MTC)			
		10	30	50	70
t_{max} (s)					
Rectus femoris	30°	81.9 ± 48.7*†	22.7 ± 10.5*	12.0 ± 4.5*	7.5 ± 1.9*‡
	60°	37.3 ± 18.6†	11.3 ± 4.7	7.0 ± 3.0	4.8 ± 1.1‡
	90°	39.6 ± 19.0†	13.1 ± 6.9	5.8 ± 0.9	4.3 ± 0.7‡
Vastus lateralis	30°	27.3 ± 14.3*	17.6 ± 7.9*	12.6 ± 2.9*	8.8 ± 2.0*
	60°	21.3 ± 14.1	11.2 ± 1.2	7.3 ± 1.0	5.8 ± 0.8
	90°	18.8 ± 2.9	11.0 ± 1.6	7.0 ± 1.2	5.2 ± 0.7
Vastus medialis	30°	40.0 ± 24.4*	24.5 ± 15.8*	15.2 ± 5.6*	10.3 ± 3.4*
	60°	19.0 ± 7.1	11.9 ± 1.9	7.9 ± 1.5	6.2 ± 0.8
	90°	18.1 ± 2.8	11.1 ± 1.7	6.9 ± 1.0	5.2 ± 1.0

* Significantly lower than 60 and 90° at all intensities and for all muscles, †Significantly greater than VL and VM at 10%MTC, ‡Significantly smaller than VL and VM at 70%MTC, $P < 0.05$, $n = 9$

of the RF muscle was 250.2 ± 59.4% of the VL and VM (averaged) muscle across the 60° and 90° knee angles. For each subject, m$\dot{V}O_2$ was also measured at the same point in time after torque onset (14.5 ± 2.0 s) for all muscle and angles. Similar to m$\dot{V}O_{2max}$, a significant main effect for muscle was found. In addition, m$\dot{V}O_2$ of the RF tended to be smaller than VM ($p = 0.069$), but not VL ($p = 0.148$).

At 10%MTC, rsEMG values were not different between muscles at the start of the contraction. However, from the start of the contraction to the time when m$\dot{V}O_2$ was measured, VL rsEMG values increased significantly at 30° and 60° knee angles, and VM rsEMG values at 30, 60 and 90° knee angle, whereas RF rsEMG values showed no change (Fig. 6).

Discussion

In the present study, isometric knee extension contractions at different intensities were compared between knee angles with respect to muscle activation (rsEMG) and muscle oxygen utilization (m$\dot{V}O_2$). The first finding of the present study was that a tendency for lower muscle activation at 30° may contribute to, but cannot completely account for the substantially lower m$\dot{V}O_2$ at 30° compared to 60 and 90° knee angles. Second, at low intensities of contraction, the slower increase in RF m$\dot{V}O_2$, coupled to the slower relative increase of RF rsEMG, suggests a less intense activation of the RF compared to the VL and VM muscles.

Muscle oxygen consumption between knee angles

The lower m$\dot{V}O_{2max}$ at 30° compared to 60° and 90° knee angles in the present study was expected and reaffirms results of an earlier study of ours (de Ruiter et al. 2005). We measured m$\dot{V}O_2$ during complete arterial occlusion and used this as a measure for energy consumption, which is a reasonable assumption as has been argued previously (de Ruiter et al. 2005).

In addition, after torque onset, at each intensity significantly more time was required to reach m$\dot{V}O_{2max}$ at 30° compared to the other two joint angles (Table 2), which is a further indication that isometric contractions are less strenuous at 30°. Moreover, by measuring m$\dot{V}O_{2max}$ for each muscle at each knee angle, the present study shows that the time at which m$\dot{V}O_{2max}$ occurred after torque onset varied greatly between muscles and knee angles. Because we were interested in comparing task demand at relative torque levels between knee angles, for each subject we compared m$\dot{V}O_2$ (and rsEMG) at the same point in time at each intensity level. This revealed that isometric knee extensions are energetically even less demanding at 30° than at 60° and 90° knee angles compared to our previous study, where only m$\dot{V}O_{2max}$ was studied (de Ruiter et al. 2005). In fact: Averaged from 10–70%MTC, m$\dot{V}O_2$ at 30° was only ~40% of the m$\dot{V}O_2$ that was needed at the 60° and 90° knee angles.

It is improbable that the lower m$\dot{V}O_2$ at 30° compared to 60° and 90° is the result of an underestimation of the MTC at 30°. As a result of our strict selection criteria of an activation level of >90% at each knee angle, voluntary activation levels were very high (Table 1). The linear extrapolation of the extra torque as a result of electrical stimulation on high intensity contractions (>70%MVC) resulted in high R^2 values. Together with the high levels of voluntary activation, this ensured an accurate estimation of the 100% value for MTC at each knee angle and consequently enabled a proper comparison of relative torques between knee angles.

During the sustained maximal attempts on day 2 maximal voluntary activation was lower at the 60°

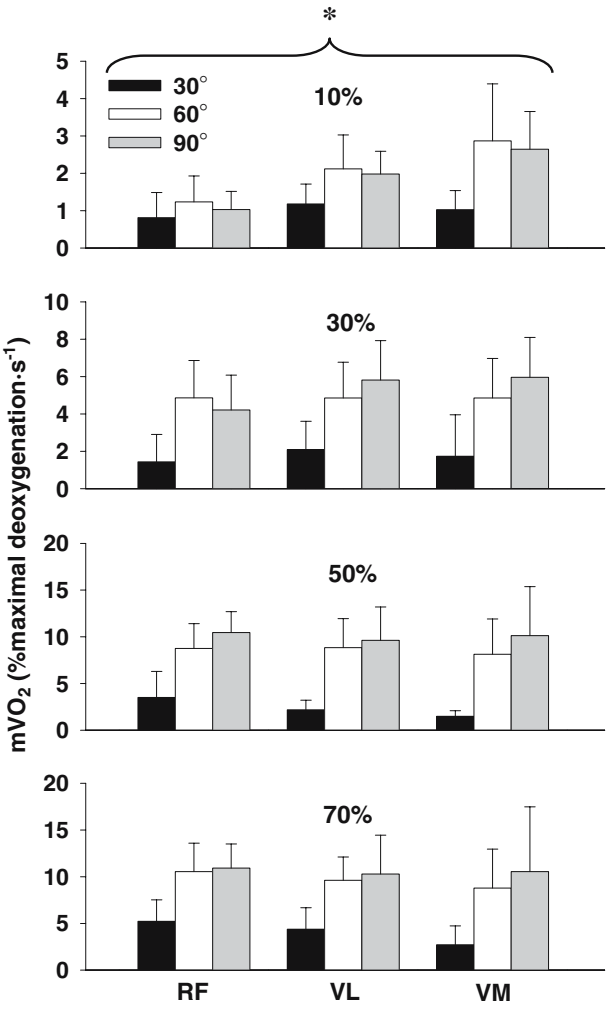

Fig. 4 Muscle oxygen consumption for the rectus femoris (RF, on the left), vastus lateralis (VL, mid) and vastus medialis (VM, right) muscle at different contraction intensities (10–70%MTC). At all intensities, mVO_2 was significantly smaller at 30° (*black bars*) compared to 60 (*white bars*) and 90° (*grey bars*) for all muscles. * At 10%MTC there was a significant main effect of muscle and mVO_2 of the RF tended to be smaller than VM ($p = 0.069$). Please note the differences in scale of the *y*-axis

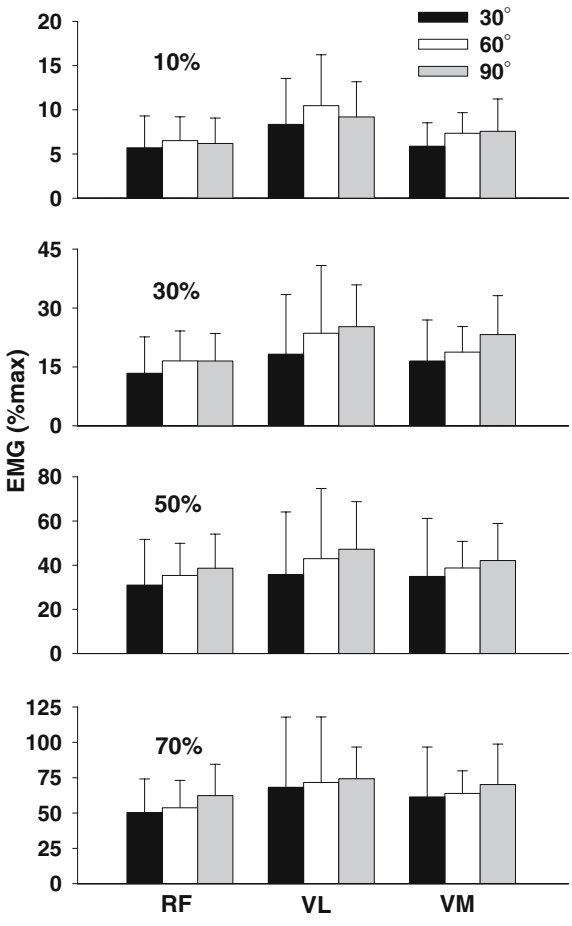

Fig. 5 Rectified surface EMG at the start of contraction. Rectus femoris (RF, on the left), vastus lateralis (VL, mid) and vastus medialis (VM, right) muscle at different contraction intensities (10–70%MTC) are shown for 30° (*black bars*), 60° (*white bars*), and 90° (*grey bars*) knee angles. Please note the differences in scale of the *y*-axis

(83.1 ± 11.7%) and 90° (74.4 ± 7.1%) knee angle. This was expected since during sustained maximal contractions initial voluntary activation is rarely 100%, as seen in the m. biceps brachii for example (Schillings et al. 2005). Surprisingly however, maximal voluntary activation was very high (96.2 ± 3.8%) at the 30° knee angle during the sustained maximal contraction, another indication that maximal contractions at this knee angle seem to be less demanding compared to 60 and 90°.

Muscle activation between knee angles

A potentiation-related relatively lower increase in muscle activation at 30° compared to the other knee angles could contribute to the lower energy consumption at the 30° knee angle. In rat muscle, twitch potentiation is inversely proportional to muscle length, the magnitude of the enhancement being greater at shorter muscle lengths (Rassier et al. 1997; Rijkelijkhuizen et al. 2005). In addition, after a fatiguing contraction in the human quadriceps muscle, peak twitch and doublet force were significantly potentiated at short (35° knee angle) but not at long (75° knee angle) muscle length (Place et al. 2005). This higher level of twitch potentiation at extended compared to more flexed knee angles has been suggested to underlie the greater endurance time at extended compared to more flexed knee angles (Place et al. 2005). Potentiation of force production, while force output remains constant, is expected to lead to a lower increase in muscle activation; motor unit firing rates may even decrease during constant force production (Klein et al. 2001). A lower increase in the

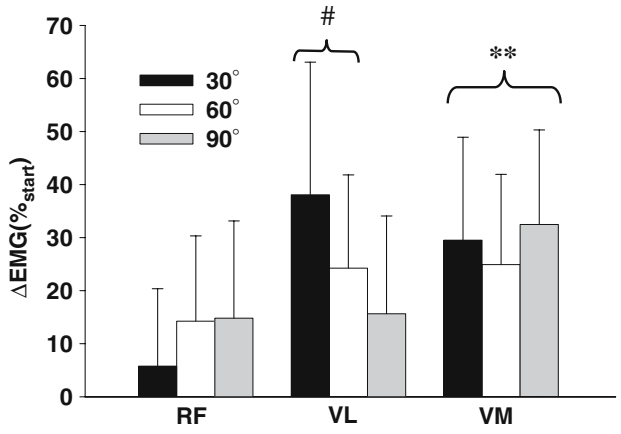

Fig. 6 Increase in rsEMG at 10%MTC of the rectus femoris (RF, on the left), vastus lateralis (VL, mid) and vastus medialis (VM, right) muscle from the start of contraction to the time mVO_2 was measured. # VL increased significantly at 30 (*black bars*) and 60° (*white bars*) knee angle. **VM increased significantly at 30°, 60°, and 90° (*grey bars*) knee angle

required muscle activation during constant torque production at 30° compared to the 60 and 90° knee angle, could in turn lead to a decrease of the activation dependent energy consumption. The latter being mainly related to calcium release and re-accumulation by the sarcoplasmic reticulum (Homsher et al. 1972). However, in the present study, there was only a tendency for rsEMG levels to be lower at 30°. The magnitude of this potential knee angle effect in each muscle (13–21%) is small compared to the knee angle effect on mVO_2 that was found (see above) to be 53–64% lower at the 30° knee angle. In addition, such an approximately 15% lower muscle activation at 30° may account for only a small fraction of the ~60% lower mVO_2 at 30° compared to 60° and 90° knee angles. The energy cost of muscle activation during maximal isometric contraction at optimum length is in the order of 30% of the total energy consumption (Homsher et al. 1972). This fraction may be somewhat higher at very low muscle lengths (de Haan et al. 1986), thus, a reduction of muscle activation of about 15%, as suggested by the strong tendency for lower surface EMG found in the present study at the 30° knee angle would, at most, account for a 5–10% lower mVO_2 at 30° compared to 60 and 90° knee angles. This leaves at least 80% of the ~60% lower energy consumption at 30° unaccounted for.

How can we account for the remaining 80% of the ~60% lower energy consumption at 30° compared to 60 and 90°? A length-dependency of energy consumption might provide an explanation. Despite reports of an unchanged energy consumption at short compared to optimum muscle length in isolated mammalian muscle (Phillips and Woledge 1992; Stephenson et al. 1989), at very low muscle lengths energy consumption did decrease compared to that found at optimum muscle length (de Haan et al. 1986; Sandberg and Carlson 1966). Using ^{31}P-MRS, Sacco et al. (1994) and Baker et al. (1992), reported similar rates of ATP use at short and optimum length of the tibialis anterior muscle in humans during contraction. This might indicate that the relative length changes in vivo may be rather small. The knee extensors most likely operate on the ascending limb of the length-tension relationship at 30°, whereas 60 and 90° knee angles will be closer to optimal muscle length (Ichinose et al. 1997; Suter and Herzog 1997).

From the above, it seems unlikely that at equal relative intensities the lower mVO_2 at 30° (short muscle length) would be the result of length-dependent lower energy consumption compared to 90° (long muscle length). The present study shows that the lower mVO_2 at 30° compared to 60 and 90° cannot be accounted for by differences in muscle activation and consequently the exact explanation remains to be established.

Muscle oxygen consumption and muscle activation at 10%MTC

At the 10%MTC level, we hypothesized that a relatively lower mVO_2 of the RF would be accompanied by a lower level of muscle activation of the RF compared to both vasti. There was a significant main effect for mVO_2 between the three muscles at the 10%MTC contraction intensity and post-hoc analysis showed a tendency ($p = 0.069$) for RF mVO_2 to be lower than VM mVO_2. These results seem to be less conclusive than those of de Ruiter et al. (2005) where RF mVO_2 was clearly lower than both vasti. This might be explained by the fact that in our study in one out of nine subjects a faster increase in, as well as a higher mVO_2 at 10%MTC was found in the RF than in the vasti at each knee angle. This accounts for the large standard deviation seen in t_{max} at 10%MTC (Table 2) and the less distinct difference in energy consumption between the RF and the VL and VM muscles, compared to our previous work (de Ruiter et al. 2005). In the present study the slower increase in RF mVO_2 (t_{max} of the RF was substantially greater than that of the VL and VM muscles) was not accompanied by less EMG activity of the RF compared to the VL and VM muscle at the start of the contraction at any of the knee angles tested (Fig. 5, top panel.). However, the increase in RF EMG from torque onset to the time mVO_2 was measured (14.5 ± 2.0 s) was less compared to the two vasti, the VL and VM muscle showed increases in EMG activity of 26 and 29%, whereas the RF muscle only showed an increase of 12% (see Fig. 6).

During low intensity isometric contractions, there are indications of less RF EMG activity compared to both vasti in some studies (Alkner et al. 2000; Clark et al. 2005), whereas in others this is not the case (Pincivero and Coelho 2000), or even the opposite (more EMG activity of the RF compared to VL and VM muscles) is seen (Rochette et al. 2003). It is not clear why these differences exist between studies; though speculation, different hip angles ranging from 90° to 120° (180° = full extension), with some authors (Pincivero and Coelho 2000) unfortunately failing to specify hip angle, may be a factor. Because the RF spans both knee and hip joint, both joint angles will influence its muscle length and thereby potentially its activation relative to the other knee extensors (see (Rochette et al. 2003). Our findings are in agreement with the slow development of the integrated EMG of the RF muscle compared to the VL and VM muscle reported by Kouzaki et al. (2003) at the 90° knee angle. Apparently, in the present study, similar levels of muscle activation of the RF, VL, and VM at the start of the contraction are not necessarily maintained further on in the contraction and it seems as if the relative contribution of the RF to total torque production decreases. Another explanation may be that the RF fatigues less quickly than the VL and VM and therefore requires a comparatively smaller increase in EMG. This dissimilar increase in EMG might lead to the considerably greater t_{max} of the RF, compared to VL and VM muscles at 10%MTC found in the present study. Whatever the mechanism for this finding, at low contraction intensities subtle differences in mVO_2 between muscles were accompanied by differences in EMG, whereas the far more pronounced difference in mVO_2 between knee angles was not accompanied by a comparable difference in EMG.

In conclusion, the present study shows that during isometric contractions (1) The tendency for lower muscle activation at 30° compared to the 60° and 90° may contribute to, but certainly cannot account for the lower muscle oxygen consumption at the 30° knee angle. (2) At low contraction intensities, the slower increase in mVO_2 of the RF seems to be due to a less intense activation of the RF compared to the VL and VM muscles.

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